Yao Lu[1,2]
Weiwei Shi[1,2]
Lei Jiang[1]
Jianhua Qin[1]
Bingcheng Lin[1]

[1]Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, P. R. China
[2]Graduate School of Chinese Academy of Sciences, Beijing, P. R. China

Received September 2, 2008
Revised November 10, 2008
Accepted November 10, 2008

Short Communication

Rapid prototyping of paper-based microfluidics with wax for low-cost, portable bioassay

Here we present a simple and low-cost production method to generate paper-based microfluidic devices with wax for portable bioassay. The wax patterning method we introduced here included three different ways: (i) painting with a wax pen, (ii) printing with an inkjet printer followed by painting with a wax pen, (iii) printing by a wax printer directly. The whole process was easy to operate and could be finished within 5–10 min without the use of a clean room, UV lamp, organic solvent, *etc*. Horse radish peroxidase, BSA and glucose assays were conducted to verify the performance of wax-patterned paper.

Keywords:
Bioassay / Microfluidics / Paper-based microfluidics / Wax
DOI 10.1002/elps.200800563

This paper presents a very simple and low-cost method to pattern microstructures with wax on paper for portable bioassay. Here, we simply use wax as a hydrophobic barrier to form well-defined millimetre-sized channels on the hydrophilic paper. The process for patterning paper with wax is very simple, which can either be done at home using a wax pen or massively produced with a wax printer. The performance was demonstrated by running multiple colorimetric assays. This assay system has the advantages of ease of use, low production cost, high portability and mobility. We believe it will be very useful for prototyping paper-based microfluidic assays and has a significant promise for low-cost monitoring of health in remote regions.

Paper-based assays have been previously used for a variety of simple diagnostic test [1, 2]. Recently, patterned paper as a paper microfluidic diagnostic platform was first proposed by Whitesides and co-workers [3–6], which demonstrated the capability to perform multiplexed assays on a piece of paper with small volume of samples and realize inexpensive on-site analysis. In their work, paper was patterned with photoresist or PDMS, which were used to form hydrophobic boundaries to direct the fluid to the test area [3–7]. However, these methods to generate patterned paper obviously suffer from relatively complex fabrication processes and higher cost of materials, which are not ideal for minimizing the cost of paper-based microfluidic device.

In this work, we presented a very simple method for patterning paper as a potential diagnostic platform for portable bioassay. Here, we simply pattern the paper with wax instead of SU-8 or PDMS to form the hydrophobic boundaries on hydrophilic paper. Physically, wax is hydrophobic, plastic (malleable) at normal ambient temperature and insoluble in water with a relatively low viscosity when melted. Patterning paper with wax rather than SU-8 and PDMS offers significant advantages, which are cheap, easy to use and convenient for printing. The schematic illustration of the processes to creat patterned paper is presented in Fig. 1A.

We patterned filter paper with wax in three different ways: (i) painting with a wax pen, (ii) printing with an inkjet printer followed by painting with a wax pen, (iii) printing by a wax printer directly. The first way is to use a wax pen to draw the desired pattern on the both sides of a filter paper (102 or 202, Hangzhou Xinhua Paper Limited, China). Then, we put the filter paper in the oven (about 150°C) for about 5 min. The melted wax will penetrate the paper to form the hydrophobic wall on the paper, allowing the liquid flowing along the edges (Fig. 1B(a)). It is noted that the whole process is very simple and only two steps are required (painting and heating). Totally, it takes 5–10 min to complete the process. In particular, the materials used include a wax pen, a ruler and a heating equipment, which are all cheap and easy to get. Thus, with the production method established, the patterned paper can be easily DIY

Correspondence: Professor Bingcheng Lin, Dalian Institute of Chemical Physics, Chinese Academy of Sciences, 457 Zhongshan Road, Dalian, P. R. China
E-mail: bclin@dicp.ac.cn
Fax: +86-411-84379650

Abbreviations: HRP, horse radish peroxidase; **TMB,** tetramethyl benzidine

***Additional corresponding author:** Professor Jianhua Qin
E-mail: jhqin@dicp.ac.cn

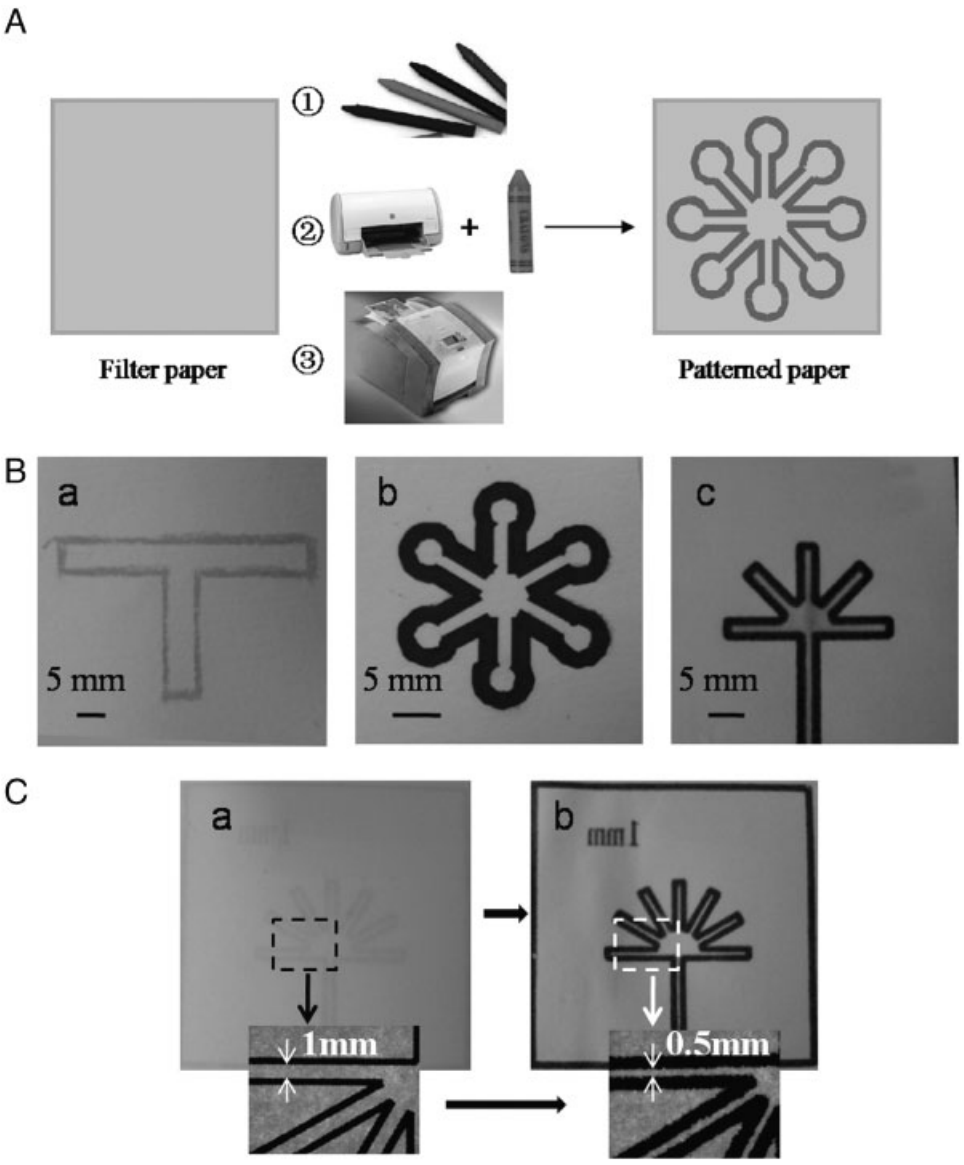

Figure 1. (A) Schematic illustration of the processes to produce patterned paper with wax; (1) hand drawing with a wax pen; (2) printing with an inkjet printer followed by painting with a wax; (3) printing with a wax printer. (B) The images of filter paper patterned with wax in three different ways: (a), hand drawing with a wax pen; (b), printing with an inkjet printer followed by painting with a wax pen; (c), printing with a wax printer directly. (C) Comparison of the back side and channel width of patterned paper produced by wax printer before and after heating in oven: (a)before heating; (b) after heating (1 mm is the width of the micro-channels).

(do it yourself) at home to realize point of care diagnosis in resource-limited regions.

The second way is to design the pattern on computer first and print it on filter paper with an inkjet printer (HP LaserJet 1000 series, USA), then paint the printed pattern with a wax pen from both sides of the paper. Finally, the wax on the paper is melted in an oven at a temperature about 150°C, allowing it to penetrate the paper to form wax wall (Fig. 1B(b)). As compared with the first one, this method is suitable for the cases when complicated pattern design is required.

The third way is to print the designed pattern on the filter paper directly using a wax printer (FUJIXEROX Phaser 8560DN, Japan; printer resolution is 2400 dpi × 2400 dpi) [8, 9]. The printing process is quite simple without the use of wax pen (the height of the wax line is about 10 μm and the thickness of the filter paper is 120 μm in our experiment). After printing, we melted the printed wax in the oven. Owing to the porous structure of the filter paper, the wax can penetrate into the paper to form well-defined microchannels on the paper. The duration time for the patterned wax on the paper to penetrate through depends on the temperature used. For example, if the paper chip was kept in an oven of 110°C, then 5 min was needed for the wax to penetrate through the paper fully. If the temperature in the oven were increased to 130°C, then the penetration process would be completed in about 30 s. Normally, the temperature used for melting the wax in Phaser 8560DN is 135°C, here we chose a relatively higher temperature (150°C) in order to shorten the fabrication time. We also kept the wax-printed paper in an oven of 60°C for 30 min and we did not observe any penetration. This indicated that the wax-patterned paper is stable and can be stored under 60°C, which is sufficient for use. The melted wax will not only

penetrate through the paper but also flow toward both sides of wax line, which are induced by the diffusion of wax and absorbability of paper. So the resulting channel width is thinner than the original channel width. And this process will come to an equilibrium in about 2 min when the temperature in the oven is 135°C. In our experiment, we found that the resulting channel width can be estimated according to the minus result between the original channel width and the original wax line width. For example, if the original channel width is 2 mm and the wax line is 1 mm, then the resulting channel width is estimated to be about 1 mm (the actual channel width obtained is 1.18 mm). After printing and heating, the patterned paper is ready for use after cooling (Fig. 1B(c)). Figure 1C shows the differences of the patterned paper before and after heating. It is obvious that the wax is able to penetrate the paper and become visual after heating from Fig. 1C(a) and (b) and the channels width was reduced from 1 mm to about 500 μm after heating. This method only requires the wax printer for direct printing, which is much easier than the previous two methods. In addition, as the printer we used can print 30 pages of A4-sized paper within 1 min, in principle, the patterned paper can be massively produced. We believe this method will be attractive to produce low-cost bioassay paper at a large scale for point of care testing.

We also tried to print the wax pattern on normal A4 office paper, however, we found that such kind of paper exhibited poor water absorbance and needed to be oxidized in oxygen plasma, or required additional surfactants in the samples to facilitate the fluid flowing.

Minimizing the quantity of the used chemical reagents reduces manufacturing costs especially with the wax-patterned method we presented here. The established wax-based methods are quite easy, fast and cheap, which can be easily accomplished out of clean room or even at home. As compared with photoresist-based patterning method proposed by Whitesides and co-workers, the wax-based patterning method we presented is rather simple. More importantly, it is quite suitable for mass production of low-cost-patterned paper, which is very attractive for practical use at a large scale. The differences and comparisons between wax-based and photoresist-based methods are listed as below:

(i) Production process: SU-8 or PDMS methods include cumbersome steps such as exposure, developing and curing, which might take hours or a day to finish, while for wax, it includes only printing and heating steps and takes only several minutes to finish.
(ii) Production speed: Wax printer we used can print 30 pages of A4-sized paper in 1 min, so the patterned paper can be massively produced in principle.
(iii) Cost: Wax is obviously much cheaper and easier to get than SU-8 or PDMS, this feature is quite important and absolutely necessary when it is put into the practical use. Meanwhile, the whole process was easy to operate without the use of a clean room, UV lamp, mask or any organic solvent.
(iv) Environmentally friendly: SU-8 or PDMS methods need the aid of organic solvent that may damage the original structure of the filter paper. And sometimes, oxygen plasma oxidation was required to render the paper back to hydrophilic. While for wax there is no such a problem.

As mentioned above, we think the method for patterning the microstructure and micro-channels with wax is novel and it represents a highly attractive alternative to stamp printing using silicone or photoresist. We believe this method will be attractive to produce low-cost bioassay paper at a large scale for point of care testing.

The common enzyme reaction was carried out to characterize the capability of this device for potential bioassay applications. The enzyme reaction between tetra-methyl benzidine (TMB) and horseradish peroxidase (HRP) is a colorimetric assay, in which TMB can be catalyzed by HRP and changed into blue color product after the reaction [10]. We first deposited 0.2 μL 5×10^{-5} mol/L HRP solution on the outlet of the straight branches alternately, allowing the reagents to dry in the air. After the addition of 10 μL TMB solution in the center of patterned paper, it was observed to disperse equally along the six flow branches. Once TMB flowed into the regions pre-spotted with HRP, the enzyme reaction started and generated blue color product. From Fig. 2A(a), we can clearly observe that the generated color product in the three wells deposited with HRP. In comparison, no color change was found in the other control wells (Fig. 2A(b), labeled with dashed lines). The results suggested there was no cross contamination between the neighboring channels, and it is the basis for realizing multiple analytes assay simultaneously.

Patterned paper with wax can be employed for biological assay by adding appropriate reagents to the test area on the

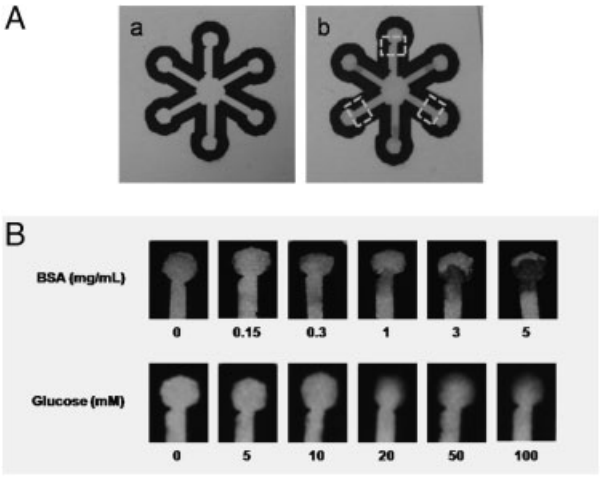

Figure 2. (A) Image results of colorimetric assay between HRP and TMB: (a), patterned paper spotted with the HRP reagent; (b), HRP reacted with TMB to produce blue color product; (c) the dashed line showed the control channels. (B) BSA and glucose assays on patterned paper with varied concentrations.

device. We demonstrated the capability by assay of glucose and protein simultaneously on the patterned paper. Glucose and protein BSA were chosen as the model analytes for verifying the performance with three duplicates for each analyte. The principles for the glucose and protein assay are based on well-developed one-step color reactions [11, 12]. The protein and glucose assays were conducted according to the procedures previously reported [3]. For glucose assay, we deposited 0.5 μL of potassium iodide (0.6 M), 0.5 μL of the mixture of HRP/glucose oxidase sequentially into the end of the branches alternately. For protein analysis, we deposited 0.5 μL of a 0.3 M citrate buffer solution (pH 1.8) in another three branches and then added 0.5 μL BPB (Amresco, USA) in 95% ethanol (3.3 mM) over the citrate buffer solution. After the necessary reagents were deposited and dried in the air, the mixture of glucose and BSA with different concentrations was added and the sample flowed along the hydrophilic channels to the test area and reacted with deposited reagents. From the results of the glucose assay and protein assay shown in Fig. 2B, we can differentiate the differences between varied concentrations.

Patterned paper has provided a new inexpensive platform for portable diagnostics assay, in particular, for assay development, which is simpler than the commonly referred microfluidic devices [13, 14]. Multiplex assays can be performed on the paper-based microfluidic platform simultaneously with minimal sample consumption. Patterning paper with wax is significantly more flexible than photoresist or PDMS in paper and offers obvious advantages such as fast and easy fabrication process (5–10 min), extremely cheap (both wax and paper are cheap and easy to obtain), no requirement for special equipments and power resources, no use of organic solvent. Thus, the wax-based micro-patterning technology will provide many opportunities to implement low-cost bioassays in a short time in remote settings. We believe it will open new possibilities and bring broad applications in clinical diagnosis, food safety inspection and environmental screening at home and resource limited regions [15, 16].

This research was supported by the National Nature Science Foundation of China (No. 20575067, 20635030 and 90713014), 863 Program, Ministry of Science and Technology of China (No. 2006AA02Z305 and 2006AA020201), 973 program, Ministry of Science and Technology of China (No. 2007CB714505 and 2007CB714507), Key Projects in the National Science & Technology Pillar Program in the Eleventh Five-year Plan Period of China (No. 2006-BAD12B03-01), and Innovation Project of Chinese Academy of Sciences.

The authors have declared no conflict of interest

References

[1] Willyard, C., *Nat. Med.* 2007, *13*, 1128–1129.

[2] Free, H. M., Collins, G. F., Free, A. H., *Clin. Chem.* 1960, *6*, 352–361.

[3] Martinez, A. W., Phillips, S. T., Butte, M. J., Whitesides, G. M., *Angew. Chem. Int. Ed.* 2007, *46*, 1318–1320.

[4] Bruzewicz, D. A., Reches, M., Whitesides, G. M., *Anal. Chem.* 2008, *80*, 3387–3392.

[5] Martinez, A. W., Phillips, S. T., Carrilho, E., Thomas, S. W., Sindi, H., Whitesides, G. M., *Anal. Chem.* 2008, *80*, 3699–3707.

[6] Martinez, A. W., Phillips, S. T., Wiley, B. J., Gupta, M., Whitesides, G. M., *Lab Chip* 2008, *8*, 2146–2150.

[7] Hardman, J. D., Slater, J. H., Reid, A. G., Lang, W. K., Jackson, J. R., Diamatrix Ltd. U.S. Patent 2003, 6,573,108.

[8] Kaigala, G. V., Ho, S., Penterman, R., Backhouse, C. J., *Lab Chip* 2007, *7*, 384–387.

[9] Maltezos, G., Garcia, E., Hanrahan, G., Gomez, F. A., Vyawhare, S., van Dam, R. M., Chen, Y., Scherer, A., *Lab Chip* 2007, *7*, 1209–1211.

[10] Jia, M., He, Z., Jin, W., *J. Chromatogr. A.*, 2002, *966*, 187–194.

[11] Peele J. D. , Jr., Gadsden, R. H., Crews, R., *Clin. Chem.* 1977, *23*, 2242–2246.

[12] Wei, Y. J., Li, K. A., Tong, S. Y., *Talanta* 1996, *43*, 1–10.

[13] Ye, N. N., Qin, J. H., Liu, X., Shi, W. W., Lin, B. C., *Electrophoresis* 2007, *28*, 1146–1153.

[14] Chin, C. D., Linder, V., Sia, S. K., *Lab Chip* 2007, *7*, 41–57.

[15] Schleicher, E., *Anal. Bioanal. Chem.* 2006, *384*, 124–131.

[16] Cunningham, D. D., *Anal. Chim. Acta* 2001, *429*, 1–18.

